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High heterogeneity of CFTR mutations and unexpected low incidence of cystic fibrosis in the Mediterranean France☆

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Abstract

In this report, we present updated spectrum and frequency of mutations of the *CFTR* gene that are responsible for cystic fibrosis (CF) in Languedoc-Roussillon (L-R), the southwestern part of France. A total of 75 different mutations were identified by DGGE in 215 families, accounting for 97.6% of CF genes and generating 88 different mutational genotypes. The frequency of p.F508del was 60.23% in L-R versus 67.18% in the whole country and only five other mutations (p.G542X, p.N1303K, p.R334W, c.1717-1G>A, c.711+1G>T) had a frequency higher than 1%. The mutations were scattered over 20 exons or their border. This sample representing only 5.7% of French CF patients contributed to 24% of CFTR mutations reported in France. This is one of the highest molecular allelic heterogeneity reported so far in CF. We also present the result of a neonatal screening program based on a two-tiered approach "IRT/20 mutations/IRT" analysis on blood spots, implemented in France with the aim to improve survival and quality of life of patients diagnosed before clinical onset. This 18-month pilot project showed an unexpected low incidence of CF (1/8885) in South of France, with only six CF children detected among 43,489 neonates born in L-R, and 13 among 125,339 neonates born in Provence-Alpes-Côte-d'Azur (PACA).

Keywords: Cystic fibrosis; Neonatal screening; CFTR mutations

1. Introduction

Cystic fibrosis (CF, MIM≠219700) remains the most common life-limiting recessive autosomal disease in Caucasian populations. CF is caused by mutations in the *Cystic Fibrosis Transmembrane Conductance Regulator* (CFTR; MIM≠602421) gene and is characterized by abnormalities of secretions of exocrine glands which lead to several symptoms of variable severity including a progressive decline in pulmonary function secondary to chronic lung infections, digestive disorders such as pancreatic insufficiency, infertility in males and elevated chloride concentration in sweat. As of January 2004, a total of 1291 mutations causing or non-causing disease have been reported to the Cystic Fibrosis Genetic Analysis Consortium (http://www.genet.sickkids.on.ca/cftr/). The frequency of these mutations differs tremendously according to the geographical and ethnic origin of patients, even in the same country [1]. The accurate knowledge of CFTR mutations has obvious interest in clinical testing, as it improves CF prevention programmes of neonatal screening, heterozygote screening in partners of CF patients or partners of carriers, and CF diagnosis in prenatal or postnatal circumstances. Reporting updated data is also crucial in population genetics, as most papers on CFTR mutation frequencies and history are compiled from the literature [2,3].

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We had previously highlighted a remarkable CFTR allelic heterogeneity in France, by contrast to other countries in Europe, and a marked difference in the frequency distribution of the most common mutations among the geographic regions in France [4]. In the current study, we have focused extensive and thorough CFTR genotyping of families with CF residing in the southern French region. The main aims of the present work were (1) to establish an updated spectrum of CFTR mutations and genotypes in the region of Languedoc-Roussillon (L-R), an area of 27,400 km^2 extending from the Pyrenean mountains to the Mediterranean sea and (2) to set up appropriate protocols and techniques to start a neonatal screening program for CF in this region. The results revealed that L-R displays one of the highest allelic heterogeneity described so far, which poses considerable challenges to the testing laboratory. Moreover, an 18-month experience of neonatal screening carried out in the two southern regions of France, L-R (2.3 million inhabitants) and Provence-Alpes-Côte-d'Azur (PACA, 4.6 millions) showed an unexpected low incidence of CF (1 in 8885) in the Mediterranean border of the country.

2. Materials and methods

2.1. CF population

We have analyzed a total of 229 Caucasian individuals affected with CF [119 males (51.96%), 110 females (48.04%) including two monozygotic female twins] from 215 families not known to be related, who were born and/or residing in L-R at the time of study. We included in this report only patients with diagnosis of classic CF based on typical clinical criteria and two positive sweat tests (cut-off, 60 mmol/l). The molecular diagnosis relied on the comprehensive scanning of exons and surrounding intronic sequences by Denaturing Gradient Gel Electrophoresis (DGGE, set up in our laboratory since 1993) followed by the sequencing of PCR products displaying altered migration pattern. In all cases, a familial study was performed to follow the segregation of alleles. The cost of CFTR scanning in our region (L-R) was covered by a special grant from the French Health Ministry.

2.2. Neonatal screening

A neonatal screening program for CF was implemented in June 2002 in L-R (2.3 million inhabitants) by means of immunoreactive trypsinogen (IRT) determinations and genetic analysis using Guthrie cards with an informed consent required for all the babies. The protocol was based on a two-tier method. Blood was taken from neonate's heel at 3 days of age and immunoreactive trypsinogen (IRT) was assayed by radioimmunological method from dried blood spots (Guthrie cards). If the IRT concentration was greater than 60 ng/ml (from June 2002 to February 2003) or 65 ng/ml (from March 2003), the same blood spot card was subjected to the genetic test. The 20 most common mutations responsible for CF worldwide were investigated by amplification refractory mutation system (ARMS) and migration on agarose gel (Kit Elucigene CF20, including mutations c.1717-1G>A, p.G542X, p.W1282X, p.N1303K, p.F508del, c.3849+10kbC>T, c.621+1G>T, p.R553X, p.G551D, p.R117H, p.R1162X, p.R334W, p.A455E, c.2183AA>G, c.3659delC, c.1078delT, p.I507del, p.R347P, p.S1251N, p.E60X). This test detected 77.9% of mutations responsible for CF in our CF population. If no mutation was found, the subjects positive at the first IRT were retested for IRT at 21 days of age (cutoff of 40 ng/ml). Newborns with two CFTR mutations and a positive sweat test (chloride concentration greater than 60 mmol/l) were referred with a definite diagnosis of CF. Infants with only one mutation or those who had no identified mutation and an IRT level at 21 days of age greater than 40 ng/ml were subjected to a sweat test. If the sweat test was negative, the baby was regarded as non-CF (carrier or no mutation detected). If the sweat test was positive, the neonate was considered as CF and the laboratory searched for CFTR mutations on a new peripheral blood sample through the DGGE scanning of CFTR exons and flanking regions. Babies with no mutation and a sweat test value higher than 40 mmol/l and babies with one mutation and a sweat test value greater than 30 mmol/l were regarded as possible CF patients and were enrolled for further clinical investigations by the Regional CF center in order to confirm or invalidate the diagnosis of CF. Newborns who were positive to the screening were enrolled by the Regional CF center for further clinical investigations and the family was offered genetic counselling. A similar screening programme was also implemented in the other Mediterranean region, Provence-Alpes-Côte d'Azur ("PACA", 4.5 million inhabitants), with our laboratory involved in CFTR mutation analysis.

2.3. Nomenclature

Gene variants, mutants and mutational genotypes were named as recommended in the Human Genome Variation Society web page (http://www.genomic.unimelb.edu.au/mdi/).

3. Results

3.1. Spectrum of CFTR mutations and genotypes responsible for CF in L-R

In 215 CF patients, a total of 75 different mutations scattered all over the CFTR gene were identified, accounting for 97.6% of CF alleles (Table 1). The main mutation, p.F508del, was present in 60.23% of chromosomes versus

Table 1
Frequencies of CFTR mutations identified in 215 patients with cystic fibrosis originating from Languedoc-Roussillon, diagnosed on a clinical basis

Mutation	Location exon/intron	No. of chromosomes (frequency %)
p.M1V	1	1 (0.23)
p.M1K	1	1 (0.23)
c.300delA	3	1 (0.23)
p.P67L	3	1 (0.23)
c.359insT	3	1 (0.23)
p.G85E	3	3 (0.70)
c.394delTT	3	1 (0.23)
p.Q98R	4	1 (0.23)
p.R117H	4	2 (0.47)
p.Y122X	4	2 (0.47)
p.Y161N	4	1 (0.23)
c.621+1G>T	intron 4	1 (0.23)
c.621+2T>G	intron 4	1 (0.23)
p.I175V	5	2 (0.47)
c.711+1G>T	intron 5	5 (1.16)
p.L206W	6	3 (0.70)
p.Q220X	6	1 (0.23)
p.L227R	6	1 (0.23)
c.1078delT	7	2 (0.47)
p.R334W	7	7 (1.63)
p.R347P	7	2 (0.47)
c.1215delG	7	1 (0.23)
c.T5	intron 8	1 (0.23)
p.D443Y	9	1 (0.23)
p.I506T	10	1 (0.23)
p.I507del	10	4 (0.93)
p.F508del	10	259 (60.23)
p.F508C	10	1 (0.23)
c.1677delTA	10	1 (0.23)
c.1717-8G>A	intron 10	1 (0.23)
c.1717-1G>A	intron 10	6 (1.40)
p.G542X	11	23 (5.35)
p.S549R	11	1 (0.23)
p.G551D	11	2 (0.47)
p.R553X	11	1 (0.23)
c1811+1.6kbA>G	intron 11	4 (0.93)
c.1812-1G>A	intron 11	1 (0.23)
p.T582I	12	1 (0.23)
p.E585X	12	2 (0,47)
c.1898+1G>A	intron 12	1 (0.23)
[c.1898+5G>A ;p.E725K]	intron 12	1 (0.23)
c.1898+73T>G	intron 12	1 (0.23)
c.2183AA>G	13	4 (0.93)
c.2184insA	13	1 (0.23)
p.K710X	13	4 (0.93)
c.2423delG	13	1 (0.23)
p.S776X	13	1 (0.23)
c.2493ins8	13	1 (0.23)
p.R792X	13	1 (0.23)
p.K830X	13	1 (0.23)
p.D836Y	14a	1 (0.23)
p.W846X1	14a	1 (0.23)
c.2711delT	14a	1 (0.23)
c.2789+5G>A	intron 14b	3 (0.70)
p.S945L	15	3 (0.70)
p.D993Y	16	1 (0.23)
c.3129del4	17a	1 (0.23)
c.3195del6	17a	1 (0.23)
c.3272-26A>G	intron 17a	1 (0.23)
[c.3395insA ;pI148T]	17b/4	1 (0,23)
p.Y1092X	17b	3 (0.70)
p.E1104X	17b	2 (0.47)
p.R1158X	19	3 (0.70)
p.R1162X	19	2 (0.47)
c.3659delC	19	1 (0.23)
c.3737delA	19	2 (0.47)
p.I1234V	19	1 (0.23)
c.3849+10kbC>T	intron 19	4 (0.93)
c.3850-1G>A	intron 19	1 (0.23)
p.G1244E	20	1 (0.23)
p.W1282X	20	2 (0.47)
p.N1303H	21	1 (0.23)
p.N1303K	21	13 (3.02)
p.Q1313X	21	1 (0.23)
c.4382delA	24	1 (023)

Mutations described for the first time by our laboratory appear in bold. Variants are described using DNA and protein designation: intronic changes, deletions, insertions and frameshifts are reported at the cDNA level (c.) and amino acid changes at the protein level (p.).

67.18% in the whole country (Fig. 1). Five other mutations were found with a relative frequency higher than 1%: p.G542X (5.35%), p.N1303K (3.02%), p.R334W (1.63%), c.1717-1G>A (1.40%) and c.711+1G>T (1.16%) (Table 1). From Fig. 1, it can be seen that mutations p.G542X, p.N1303K, p.R334W and c.711+1G>T are more common in Mediterranean areas than in the whole country. Twenty-two mutations found on 2–4 chromosomes and 47 mutations found on only one chromosome accounted for 13.95% and 10.93% of CF alleles, respectively. Overall, 24/75 mutations (32%) were described by our laboratory for the first time (CF Genetic Analysis Consortium web page) (Table 1). Ten alleles (2.33%) remained uncharacterized after complete scanning of the whole 27 exons/flanking sequences and additional screening for three common mutations (c.1811+1.6kbA>G, c.3849+10kbC>T, allele 5T in IVS8) and a large deletion (CFTR del 2,3).

The 75 detected mutations generated 88 different mutational genotypes. Three genotypes were responsible for approximately half the cases of CF: p.F508del+p.F508del (39.06%), p.F508del+p.G542X (6.51%) and p.F508del+p.N1303K (3.26%). By contrast, these genotypes accounted for 47.75%, 3.4% and 2.7%, respectively, in a sample of 3,170 CF patients who had been genotyped in France in 2000 [4]. 42.32% of CF patients in the present study carried one p.F508del associated with another mutation and 16.75% carried two other mutations. In one patient (0.46%), no causative mutation was found despite extensive scanning. A diagnosis of CF occurred in 87.4% of all patients before their 10th birthday (with 60.74% diagnosed before 1 year of age). Four patients were diagnosed at age 30, 32, 34 and 41 years and the extreme age at diagnosis was 70 years, in a male who was diagnosed initially for bronchiectasis and was carrying a 5T allele in trans of a novel missense T582I. 61.3% of patients homozygous for p.F508del and

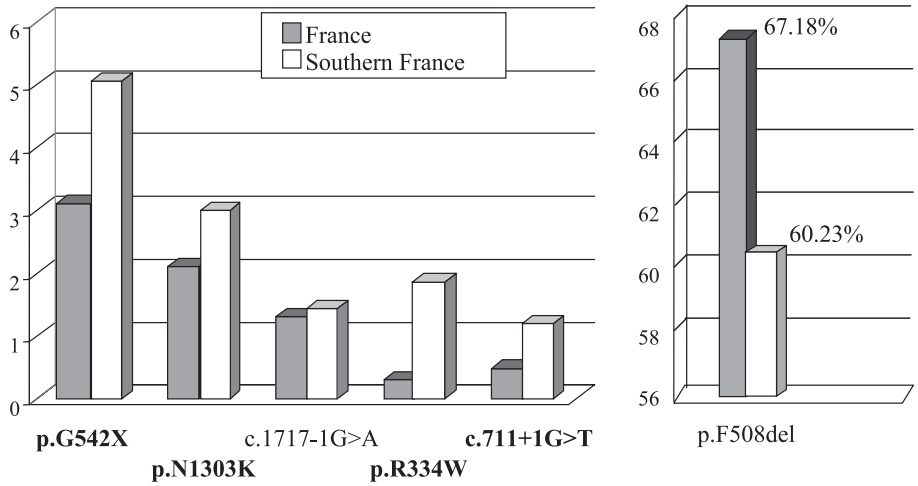

Fig. 1. Distribution of the most common mutations (frequency>1%) in southern France and in the whole country (in bold, mutations typical of Mediterranean areas).

54.4% of patients compound heterozygotes for a severe mutation were diagnosed before 6 months, whereas 86.5% of patients carrying either a severe or mild allele in combination with a mild or unknown mutation were diagnosed after 6 months (Fig. 2).

3.2. Neonatal screening in the two Mediterranean regions of France: L-R and PACA

Between June 2002 and December 2003, 168,828 newborns were screened for CF in L-R and PACA. The IRT-1 concentrations were higher than the cutoff in 1299 samples (0.77%) and were followed by the genetic test which detected 11 cases with two mutations: eight were homozygous for the mutation p.F508del, two were compound heterozygotes (p.I507del+p.R334W and p.G542X+p.R117H) and one was homozygous for the mutation p.R117H associated with the 7T allele in the intron 8 polythymidine sequence (IVS8) (Table 2).

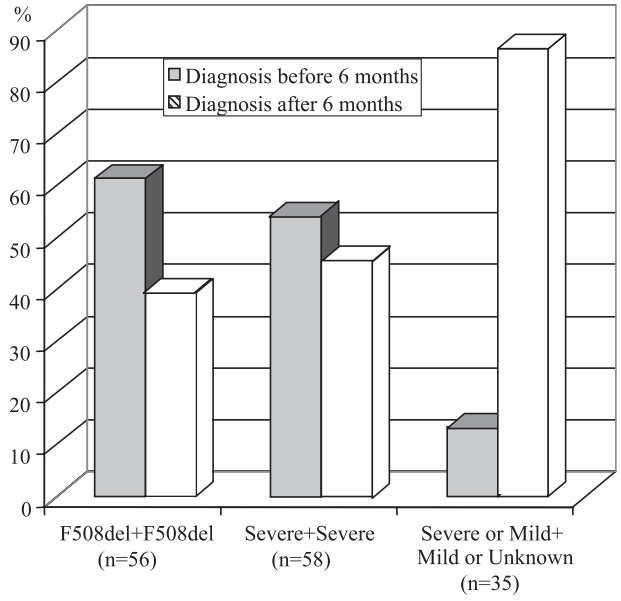

Fig. 2. Age at diagnosis of the CF patients in Languedoc-Roussillon according to genotype.

Seventy-nine newborns in whom only one mutation was identified were referred to the regional CF center. Among them, six cases were diagnosed as having CF because of a positive sweat test. The second mutation responsible for CF was detected by DGGE scanning followed by sequencing of PCR products displaying an abnormal pattern. Six rare mutations were identified: c.3007delG, c.2622+1G>A and c.3850-1G>A in trans of the mutation p.F508del, c.2380del8

Table 2
Genotypes identified by newborn screening in 19 affected babies

IRT (ng/ml)	Genotypes
118	[p.F508del]+[p.F508del][a]
163	[p.F508del]+[p.F508del][a]
>130	[p.F508del]+[p.F508del][b]
>130	[p.F508del]+[p.F508del][b]
>130	[p.F508del]+[p.F508del][b]
155	[p.F508del]+[p.F508del][a]
166	[p.F508del]+[p.F508del][a]
109	[p.F508del]+[p.F508del][a]
110	[p.F508del]+[p.F508del][a]
136	[p.F508del]+[c.3007delG][a]
160	[p.F508del]+[c.2622+1G>A][a]
129	[p.F508del]+[c.3850-1G>A][a]
151	[p.G542X]+[c.2380del8][a]
131	[c.1078delT]+[p.K710X][a]
>130	[p.I507del]+[p.R334W][b]
75	[p.G542X]+[p.R117H ;c1342-6 T7][b]
MI	[p.E1104X]+[p.E1104X][b]
84	[p.R117H; c1342-6 T7]+[p.R117H; c1342-6 T7][a]
99	[c.2183AA>G]+[p.Q220X][a]

IRT: Immunoreactive trypsinogen (cutoff: 65 ng/ml).
MI: Meconium ileus.
[a] Babies born in PACA.
[b] Babies born in LR.

in trans of p.G542X, p.K710X in trans of c.1078delT and p.Q220X in trans of c.2183AA>G (Table 2).

In 72 cases out of 1280, the sweat test was negative and the babies were regarded as heterozygotes (frequency 1/17.7). The mutation was p.F508del (*n*=47), p.G542X (*n*=5), p.N1303K (*n*=4), p.G551D (*n*=2), p.R334W (*n*=2), c.1717-1>A (*n*=1), p.I507del (*n*=1), p.R1162X (*n*=1), [p.R117H;IVS8-T7] (*n*=8) or [p.R117H;IVS8-T5] (*n*=1). One case with borderline sweat test value (42 mmol/l) and genotype p.N1303K+unknown will be further investigated.

A neonate with IRT above 130 ng/ml at 3 days of age was not referred to the molecular laboratory because the parents refused consent. This child was later detected as being affected with CF on the basis of clinical symptoms (ileal obstruction and bowel perforation) then positive sweat tests (115 and 125 mmol/l). After complete DGGE scanning followed by the sequencing of exon 17b, this neonate born from consanguineous parents of North African descent was found to be homozygous for the mutation p.E1104X. Another case presented with meconium ileus at birth and was diagnosed clinically; a blood sample was directly referred at 7 days of age to the genetic laboratory and homozygosity for p.F508del was found. A first cousin of the mother was affected with CF, but cascade genetic screening had not been proposed to the relatives.

All other 1206 children with IRT-1 positive values and no mutation detected were retested for blood IRT at 21 days of age. The babies with positive IRT-2 were referred to the regional CF center and, at this time, all sweat tests done were negative.

3.3. Refined risk calculations

The neonatal screening program implemented in Southern France allowed the identification of six CF-affected children among 43,489 newborns screened in L-R from June 2002 to December 2003 and 13 CF-affected children among 125,339 newborns screened in PACA from April 2002 to December 2003. The incidence of CF in populations living in the two French Mediterranean regions is therefore only 1 in 8885 live births (Fig. 3). According to these data, the CF allele frequency is 0.0106, corresponding to an expected CF carrier frequency of 1 in 47. Given the mutation detection rate of 97.6% after complete DGGE scanning of CF sequences and taking into account the corrected incidence of CF after neonatal screening, the residual risk of carrying a mutation or of having an affected child after a negative mutation test can be calculated as indicated in Table 3.

4. Discussion

4.1. Increased diversity of CFTR mutations in Languedoc-Roussillon (L-R)

This study shows that the spectrum of CF-causing mutations observed in L-R is different from the one reported for the entire country [4]. The proportion of p.F508del is lower than that observed in the whole country and some mutations found with relative frequency higher than 1% in

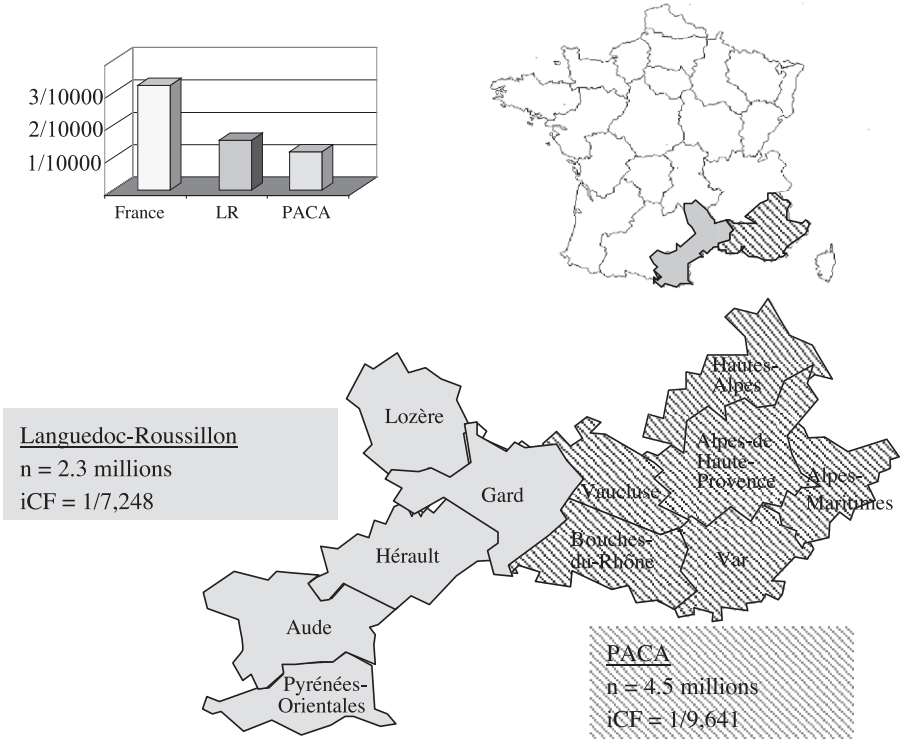

Fig. 3. Incidence of cystic fibrosis (iCF) in the two Mediterranean regions of France.

Table 3
CF-related risk after mutation analysis for carrier status in LR and PACA as modified by neonatal screening data

Percentage of CF mutations detectable	Carrier risk (Z)[a] for individuals with negative test		Risk of CF in offspring for couples tested			
			One parent positive ($Z/4$)		Neither parent positive ($Z^2/4$)	
	French[b]	LR/PACA[c]	French	LR/PACA	French	LR/PACA[g]
Not tested	1/30	1/47	Not applicable	Not applicable	1/3500	1/8885
OLA 31 mutations[d]	1/171	1/243	1/684	1/972	1/116,964	1/236,196
Elucigene CF20[e]	1/153	1/201	1/5612	1/804	1/93,636	1/161,604
Complete scanning[f]	1/1219	1/1920	1/4876	1/7680	1/5,943,844	1/14,745,600

[a] $Z=q(1-a)/(1-aq)$ with q=carrier frequency in a given population and a=sensitivity of the test, i.e. proportion of mutant CFTR alleles in a given population that can be identified with the test.
[b] Mean frequency for CF carriers in France.
[c] Mean frequency for CF carriers in LR and PACA, based on the first 18 months of CF neonatal screening.
[d] a=81% in LR-PACA and 83% in France.
[e] a=77% in LR-PACA and 81% in France.
[f] a=97.5% in LR-PACA and in France (complete DGGE scanning of CFTR exons and flanking sequences).

other French regions, such as c.394delTT and p.R553X in Northern France, c.1078delT, p.G551D and p.W846X in Brittany, or c3905insT in Eastern France [4] were found at very low rates in South. The L-R region showed one of the highest allelic heterogeneity reported so far, with 75 different mutations identified in 215 CF families. By comparison, only 80 different mutations were found in a recent study of 437 CF patients extensively genotyped by DGGE in Greece [5]. In Southern Italia, searching for only 13 mutations covered 85% of CF alleles in a sample of a similar size as ours [6].

L-R is a region of 2.3 million inhabitants, with an average annual birth cohort ranging from 29,000 to 30,000 newborns. In the course of the last century, the demographics of L-R has been unstable and ethnic mixing happened. The last return of population (1999) showed that 126,000 (5.7%) individuals were "French with recent foreign origin" and 131,000 (5.9%) were not French; including Moroccan (43,500), followed up by Spanish (23,600), Algerian (14,000), Portuguese (9000), Italian (4000), Turkish and Tunisian (more than 2000 in each group) and other populations (>30,000 European, African and Asian). These data were confirmed in a recent report on HFE mutations [7] where the author could study the ethnic background in a sample of 1272 newborns obtained from four main hospital maternity of L-R. Among them, only 43.5% had four grandparents of French origin and 14.8% had all four grandparents originating from North Africa. The remaining group included babies originating from various European, African or Asian countries. This important population admixing could explain the lower frequency of CF among neonates but cannot explain the higher allelic heterogeneity in this region. According to a recent study, the major factor that contributed to the CF mutation diversity is the size of ancestral population [3]. It is believed that at the postglaciation phase and later during the Neolithics, population expansion has started earlier, and then expanded much faster and more extensively along the Mediterranean shores than in northern parts of Europe, presumably due to more favourable living conditions [3].

As reported by many laboratories, some mutants (2.4% of alleles) remain uncharacterized after screening of the whole coding and flanking CFTR sequences. They might be located in regions not investigated (promoter, 3′ untranslated or intronic regions). In addition, although DGGE is highly sensitive and specific, we cannot exclude that some nucleotides residing in higher melting domains have been missed. Heterozygous deletions, insertions or inversions spanning a complete exon or more would not be detected by standard PCR-based scanning methods.

4.2. Improved neonatal screening

From June 2002, we used a commercially available kit including the 20 most frequent mutations worldwide (Elucigene CF20). This kit has a detection rate of 77.9% in L-R versus 81% in France. Extensive mutation analysis in L-R was essential to know the spectrum of CFTR sequence anomalies and to design a kit with a better detection rate. A new "French kit" with 10 additional mutations is in production and this panel will include mutation subsets shown to be frequent in some areas and/or populations. In southern France, 4 of these 10 additional mutations have a relative frequency above 0.5%: c.711+1G>T (1.16%), c.1811+1.6kbA>G (0.93%), c.2789+5G>A (0.70%) and p.Y1092X (0.70%). The panel of 30 mutations (c.1717-1G>A, p.G542X, p.W1282X, p.N1303K, p.F508del, c.3849+10kbC>T, c.621+1G>T, p.R553X, p.G551D, p.R117H, p.R1162X, p.R334W, p.A455E, c.2183AA>G, c.3659delC, c.1078delT, p.I507del, p.R347P, p.S1251N, p.E60X, p.Y1092X, c.394delTT, c.1811+1.6kbA>G, c.3272-26A>G, c.2789+5G>A, c.3120+1G>A, c.711+1G>T, p.G85E, p.Y122X, p.W846X) should account for 83.32% of the CF alleles in L-R and 84.25% in the whole country.

4.3. Incidence of CF in southern France

So far, CF was expected to affect about 1 in 3500 individuals in France (French National CF Observatory).

However, our first 18-month experience in neonatal screening showed that of the 43,489 births in L-R, six children with CF were identified, giving an unexpected low incidence of CF at birth of 1/7248. We obtained similar figures in the region PACA, with 13 cases (1/9641) diagnosed as CF among 125,339 neonates screened during 20 months (from April 2002 to December 2003). By contrast, Brittany, a region of 2.8 million inhabitants of mostly Celtic origin where CF is very common, presents one of the highest birth prevalence of CF observed throughout the world [8]. Indeed, the neonatal screening program implemented in Brittany 13 years ago identified 123 CF-affected children among 349,072 newborns screened between January 1992 and December 2001; the prevalence of CF at birth was therefore 1/2838 over a 10-year period in this part of the country [9].

The high allelic heterogeneity in our region could be responsible for milder forms of CF with normal neonatal IRT concentrations that could contribute to find a very low incidence in neonatal screening. This hypothesis is in agreement with data of the French National CF Observatory which showed that 50% of children were diagnosed before 4 months of age [10]. In the sample of 215 CF patients from L-R, the age at diagnosis was reported in 150 files and only 46.6% were diagnosed before 6 months. These data could confirm the hypothesis of milder or different phenotypes in Mediterranean regions. Similar data were reported in a recent study on phenylketonuria in which the author showed a high frequency of severe PKU in central and eastern European countries and a high proportion of severe mutations and milder forms in Mediterranean regions [11].

4.4. Hypertrypsinemia and CF heterozygotes

From 18 months of neonatal screening, an incidence of CF of 1 in 8885 live births leads us to an estimated carrier frequency of 1/47 (2.12%) assuming Hardy–Weinberg equilibrium. Over the same period, 72 children carrying one CFTR mutation were detected among 1280 neonates with positive IRT (1/17.7=5.65%), i.e., 2.7 times greater than the expected frequency. These data are in agreement with previous neonatal screening programs that have reported the identification, among neonates with hypertrypsinaemia, of a CF carrier frequency three times higher than expected in the general population [12–14]. At this time, the underlying mechanism of selection of carriers by the neonatal programme remains obscure. Some of these children with hypertrypsinaemia, only one mutation detected and a borderline sweat test may present atypical CF (bronchiectasis, congenital absence of the vas deferens, pancreatitis, etc.) and it has been recently suggested that these patients should be reevaluated to avoid late diagnosis [13]. Assessment of chloride conductance in respiratory and intestinal tissues by determination of nasal potential difference and intestinal current measurements may represent a useful diagnostic adjunct to precise the status of these babies [15]. It has been proposed that children who may later in life develop moderate or minimal CF be regarded as “pre-CF” [16]. It would be of great interest to know the benefit and the psychological impact of a clinical long-term follow-up in infant with mild or atypical CF with regard to a possible prevention of a hypothetic chronic lung disease in adulthood.

We found the frequency of mutation R117H (12.16%) higher in the heterozygous neonates than in the CF population in France (0.16%) or in L-R (0.47%). R117H-7T is associated with a broad phenotypic range, from CF with suppurative lung disease to no clinical disease. Extended follow-up will be required to determine whether clinical manifestations are associated with mild CFTR mutations. One can wonder whether newborn screening should be confined to classical mutations associated with severe disease or whether it should be enlarged in order to diagnose mild or atypical forms of the disease and to help in the knowledge of rare or phenotypically variable mutations.

4.5. Refinement of risks for accurate genetic counselling

Risk calculations are usually based on the estimated 1/2500 mean incidence of CF in Caucasian populations. The results of our pilot neonatal screening revealed that incidence of CF at birth in Mediterranean areas is about 1/9000. This information will have to be taken into account by genetic counsellors charged with providing couples with information concerning residual risks, particularly in the context of couples where a bowel echogenicity is detected during ultrasonography examination or couples with a partner related to a CF patient. In France, neonatal screening for genetic disease is intended to reduce childhood morbidity and mortality through early identification and treatment of affected infants [17,18] although it is still a matter of debate [19]. It is not primarily intended to offer prenatal screening of subsequent pregnancies in couples with an affected child. Neonatal screening in France does not include any information on the ethnic or geographic origin of parents, so that it is impossible to accurately calculate residual risks of CF in the different ethnic communities living in the country.

On the whole, regarding the large CFTR allelic heterogeneity in Southern France, it is important to continue to retest IRT at 21 days in babies without mutations and an IRT at 3 days of age greater than 100 ng/ml. In conclusion, this study demonstrates that, even within a single country, it is desirable to have a detailed knowledge of specific regional CF-causing mutations if we want to offer the most efficient screening strategy.

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